The mechanism of RNA recognition by KH-domain proteins KhpA and KhpB from *Streptococcus pneumoniae*

RNA-binding proteins participate in the mechanisms of control of gene expression, which are dependent on regulatory RNAs. In Gram-negative bacteria, such as *Escherichia coli* and *Salmonella enterica*, this role is fulfilled, among others, by proteins Hfq and ProQ. The roles of these proteins have been widely studied, and it is known that by binding RNAs they affect RNA lifetimes, and also that they help regulatory RNAs to find mRNAs that they control. On the other hand, very little is known about RNA-binding proteins, which cooperate with regulatory RNAs in Gram-positive bacteria, such as *Streptococcus pneumoniae*.

It has been recently found that there are two RNA-binding proteins, named KhpA and KhpB, that are present in many Gram-positive bacteria, but not in Gram -negative ones. Interestingly, in almost all of these bacterial species KhpA and KhpB are present together. It was found that in *Streptococcus pneumoniae*, and also in other species, KhpA and KhpB bind the same RNAs. This suggests that they cooperate with one another. Importantly, it was also found that they participate in the regulationl of the process of cell division, because the deletion of their genes changed the shape of cells of *S. pneumoniae*. The process of cell division is essential for bacterial multiplication, and, hence, it is targeted by many antibiotics. *S. pneumoniae* bacteria are important for human health, because they are a frequent cause of infections of upper respiratory tract, such as pneumonia, sinusitis, and otitis media.

KhpA and KhpB have in their structure RNA binding domains. In KhpA it is a single KH domain, while in KhpB there are two domains: KH domain and R3H. Such domains are also present in other RNA-binding proteins in bacteria and in higher organisms.

Very little is known about how KhpA and KhpB recognize RNAs, and why both of them have to participate in this process. To better understand what is the mechanism of action of KhpA and KhpB, in this research project we plan to find out what features are shared by RNA molecules, which are bound by KhpA and KhpB in *S. pneumoniae*. Explaining that may help us to better understand why KhpA and KhpB bind RNAs. Then we will analyze how KhpA and KhpB bind to one another, and to RNA. In particular, we will want to explain why KhpA and KhpB have to cooperate to bind RNA molecules, and also if they cooperate in the same way, when they are binding different RNAs. We will also want to find out what amino acid residues of KhpA and KhpB directly contact RNA, and what properties of the structures of KhpA and KhpB enable them to discriminate between RNAs. Finally, we will also study if KhpA and KhpB help regulatory RNAs of *S. pneumoniae* to bind to mRNA molecules.

In summary, we expect that the studies planned in this project will allow to elucidate how KhpA and KhpB, which belong to a new family of RNA binding proteins, contribute to the biological processes dependent on RNA molecules. Because KhpA and KhpB participate in the process of cell division in *S. pneumoniae* the conclusions of the planned studies may also provide new insights into the regulation of this important mechanism, which may be useful for the design of new strategies of controlling bacterial growth.